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DERWENT-WEEK: 200748

TITLE: New N-acyl homoserine lactone acylase qsbA gene encoding a QsbA protein, useful for modulating N-acyl homoserine lactone signaling activity, or for controlling a bacterial disease in a mammal or a plant

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DE 60219077E	January 23, 2002	2002DE-619077	
DE 60219077T2	January 23, 2002	2002DE-619077	
EP 1470221A1	January 23, 2002	2002EP-715955	
EP 1470221B1	January 23, 2002	2002EP-715955	
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EP 1470221B1	January 23, 2002	2002WO-SG00011	
DE 60219077E	January 23, 2002	2002WO-SG00011	
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CIPP	C12 N 9/80	20060101

ABSTRACTED-PUB-NO: WO 03068951 A1
BASIC-ABSTRACT:

NOVELTY - An N-acyl homoserine lactone acylase qsbA gene and protein composition (I), comprising:

(a) a defined sequence of 3743 base pairs (bp) (N1) given in the specification, nucleotides 1234-3618 of N1, or its fragment or substantially homologous variant; or

(b) a defined peptidic sequence of 794 amino acids (P1) given in the specification, or its fragment, subunit or substantially homologous variant, encoded by the nucleic acid of (B) (respectively), is new.

DESCRIPTION - INDEPENDENT CLAIMS are also included for:

(1) modulating N-acyl homoserine lactone (AHL) signaling activity, comprising contacting the AHL with the N-acyl homoserine lactone acylase qsbA protein;

(2) a transgenic plant or non-human animal harboring a nucleic acid consisting of nucleotides 1234-3618 of the sequence of N1, or its fragment or substantially homologous variant; and

(3) controlling a bacterial disease in a mammal or a plant, comprising administering, to the mammal or plant, the N-acyl homoserine lactone acylase qsbA protein of (I), or nucleotides 1234-3618 of the sequence of N1, or its fragment or substantially homologous variant and its peptide product (the expression of pathogenic genes of the bacteria are regulated by AHL signals).

Gene Therapy.

USE - The compositions (I), nucleic acids and proteins are useful for modulating N-acyl homoserine lactone signaling activity, or for controlling a bacterial disease in a mammal or a plant.

ABSTRACTED-PUB-NO: WO 03068951 A1
EQUIVALENT-ABSTRACTS:

BIOTECHNOLOGY

Preferred Composition: The composition of matter may also comprise a peptidic sequence comprising amino acids 36-271 or 233-794 of the sequence of P1. The composition of matter inactivates N-acyl homoserine lactone.

Preferred Method: In controlling a bacterial disease in a mammal, the mammal is preferably a human. Alternatively, controlling a bacterial disease in a plant comprises using any bacterial species containing the (I) comprising nucleotides 1234-3618 of the sequence of N1, or its fragment or substantially homologous variant.

Preparation: The nucleic acids and proteins of (I) may be produced via standard methodologies.

SPECIFIC GENES

N-acyl homoserine lactone (AHL) acylase genes from Ralstonia sp. XJ12B, termed qsbA genes, which encode QsbA proteins possessing AHL inactivating activity, are claimed.

A bacterial biofilm sample was collected from a water treatment system and screened to isolate N-acyl homoserine lactone (AHL) inactivation bacterial strains. Two bacterial isolates from the biofilm sample with distinct phenotypes, XJ12B and XJ12A, were found to possess the ability to inactivate AHL, with XJ12B showing stronger enzyme activity. A cosmid library of 1600 clones was constructed in Escherichia coli with the genomic DNA of Ralstonia sp. strain XJ12B. The E. coli transfectants were screened for AHL inactivation activity using oxooctanoyl-L-homoserine lactone (OOHL) as the substrate. Only a single clone (p13H10) was identified as showing AHL inactivation activity. To subclone the gene encoding the detected activity, cosmid DNA from the positive clone p13H10 was partially digested with Sau3A and fused into BamH1 digested cloning vector pGEM-3Zf +. The plasmids were ligated and transformed into E. coli, and the E. coli were assayed for the ability to inactivate AHL. Clone

p2B10, which contains a 4-kb insert, had AHL inactivation activity. The plasmid clone p2B10 containing the qsbA gene was used as a template. The 4-kb fragment from clone p2B10 was completely sequenced showing a sequence of 3743 bp fully defined in the specification. The longest open reading frame encodes 794 amino acids fully defined in the specification, with a predicted molecular weight of 85373 Daltons.

DERWENT-CLASS: B04 C06 D16 P13 P14
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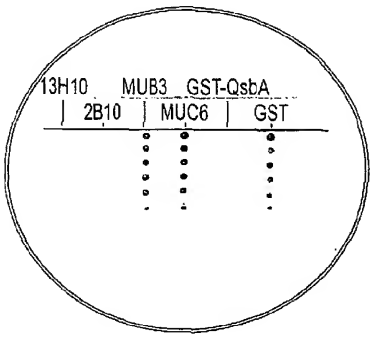
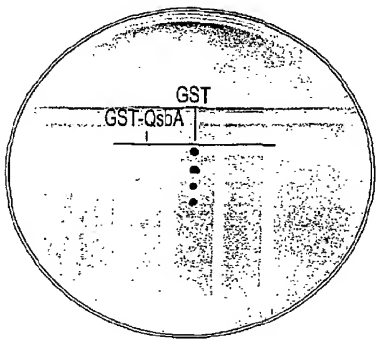
(54) Title: RALSTONIA AHL-ACYLASE GENE





(57) Abstract: This invention provides a gene, *qsbA*, which encodes a protein useful for inactivating certain bacterial quorumsensing signal molecules (N-acyl homoserine lactones) which participate in bacterial virulence and biofilm differentiation pathways. This gene was isolated from *Ralstonia sp.*, strain XJ12B. The invention also provides the QsbA protein, which possesses N-acyl homoserine lactone inactivating activity.

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RALSTONIA AHL-ACYLASE GENE

BACKGROUND OF THE INVENTION

Field of the Invention

[0001] This invention pertains to the field of molecular biology. In particular, the invention relates to an N-acyl homoserine lactone acylase gene from *Ralstonia sp.* XJ12B.

Description of the Background Art

[0002] N-acyl homoserine lactones (AHLs), also known as autoinducers, are widely used quorum sensing signal molecules in many Gram-negative bacteria. These compounds regulate certain classes of target genes in bacteria, such as virulence genes or biofilm differentiation genes. Generally, quorum sensing molecules are highly conserved and share an identical homoserine lactone moiety. The length and structure of their acyl side chains are different, however. Although the target genes regulated by AHLs in different bacteria species are varied, basic mechanisms of AHL biosynthesis and gene regulation are conserved among different bacterial species.

[0003] The general feature of AHL-mediated gene regulation is that it is cell population dependent (quorum sensing). Bacteria secrete AHLs into the environment; extracellular concentration of AHLs increases as bacterial cell populations grow. When AHL accumulates to a threshold extracellular concentration, the expression of certain sets of target genes are triggered in the bacteria.

[0004] Bacteria using these signals release, detect and respond to the accumulation of AHL signal molecules for synchronizing expression of a particular sets of genes and coordinating cellular activities within the bacterial cell population. AHLs are involved in regulation of a range of biological functions, including bioluminescence in *Vibrio* species (13, 4), Ti plasmid

conjugal transfer in *Agrobacterium tumefaciens* (31), induction of virulence genes in *Burkhholderia cepacia*, *Erwinia carotovora*, *Erw. chrysanthemi*, *Erw. stewartii*, *Pseudomonas aeruginosa*, and *Xenorhabdus nematophilus* (3, 6, 12, 17, 19, 22, 23, 24, 26), regulation of antibiotic production in *P. aureofaciens* and *Erw. carotovora* (6, 26), swarming motility in *Serratia liquifaciens* (14) and biofilm formation in *P. fluorescens* and *P. aeruginosa* (1, 8). In many other bacterial species the relevant biological functions controlled by AHLs remain to be investigated (2, 5, 11).

[0005] A number of plant, animal and human bacterial pathogens use AHL quorum-sensing signals to regulate expression of pathogenic genes and aid in the formation of biofilms. Therefore, AHL quorum-sensing signal molecules are group of molecular targets for genetic and chemical manipulations since disruption of these signaling mechanisms can prevent or reduce the ability of these bacteria to infect plant and animal tissues or to form biofilms.

[0006] The gene encoding an AHL-inactivation enzyme (AiiA) from a Gram-positive bacterium (Bacillus strain 240B1) has been cloned (9). AiiA (also known as AHL-lactonase) inactivates AHL activity by hydrolyzing the lactone bond of AHLs (10). Expression of *aiiA* in transformed *Erw. carotovora* (a pathogenic strain which causes soft rot disease in many plants) significantly reduces the release of AHL, decreases extracellular pectrolytic enzyme activities, and attenuates pathogenicity on potato, eggplant, Chinese cabbage, carrot, celery, cauliflower, and tobacco (9). Transgenic plants expressing AHL-lactonase showed a significantly enhanced resistance to *Erw. carotovora* infection and delayed development of soft rot symptoms (10). AHL-inactivation mechanisms appear to be widely distributed. For example, a bacterial isolate of *Variovorax paradoxus* was reported to use AHL molecules as its energy and nitrogen sources, indicating the possible presence of AHL-degrading enzymes (18).

[0007] Further methods to counteract AHL-mediated plant, animal and human disease and plant pathogen virulence by interfering with bacterial intercellular communication would be highly desirable.

SUMMARY OF THE INVENTION

[0008] Accordingly, in this study, the cloning and characterization of a gene encoding an AHL-acylase from a bacterial isolate *Ralstonia* sp. JX12B is reported.

[0009] In one embodiment, the invention provides a composition of matter which comprises a nucleic acid according to SEQ ID NO: 1. In another embodiment, the invention provides a composition of matter which comprises a nucleic acid selected from the group consisting of nucleotides 1234-3618 of SEQ ID NO: 1, a fragment thereof and a substantially homologous variant thereof.

[00010] In yet a further embodiment, the invention provides a nucleic acid according to claim 2 which comprises nucleotides 1234-3618 of SEQ ID NO: 1.

[00011] In yet a further embodiment, the invention provides a composition of matter which comprises a peptidic sequence selected from the group consisting of a peptidic sequence according to SEQ ID NO: 2, a fragment thereof and a substantially homologous variant thereof.

[00012] In yet a further embodiment, the invention provides a composition of matter which comprises a peptidic sequence encoded by a nucleic acid selected from the group consisting of nucleotides 1234-3618 of SEQ ID NO: 1, a fragment thereof and a substantially homologous variant thereof.

[00013] In yet a further embodiment, the invention provides a composition of matter which comprises a peptidic sequence selected from the group consisting of SEQ ID NO: 2, a fragment thereof, a subunit thereof and a substantially homologous variant thereof, such as a peptidic sequence according to SEQ ID NO: 2, a peptidic sequence comprising amino acids 36-217 233-794**[?]** of SEQ ID NO: 2 or a peptidic sequence comprising amino acids 233-794 of SEQ ID NO: 2.

[00014] In yet a further embodiment, the invention provides a composition of matter as described above which inactivates AHL.

[00015] In yet a further embodiment, the invention provides a method of modulating AHL signaling activity which comprises contacting said AHL with a composition of matter as described above.

[00016] In yet a further embodiment, the invention provides a transgenic plant or non-human mammal harboring a nucleic acid as described above.

[00017] In yet a further embodiment, the invention provides a method of controlling a bacterial disease in a mammal which comprises administering to said mammal a composition of matter as described above, wherein the expression of pathogenic genes of said bacteria are regulated by AHL signals.

[00018] In yet a further embodiment, the invention provides a method of controlling a bacterial disease in a plant which comprises administering to said plant a composition of matter as described above, wherein the expression of pathogenic genes of said bacteria are regulated by AHL signals.

BRIEF DESCRIPTION OF THE DRAWINGS

[00019] Figure 1 is a photograph showing AHL inactivation bioassay results for bacterial cultures and bacterial proteins from the indicated bacterial clones. Figure 1A shows the results of a bioassay with bacterial cultures of *E. coli* DH5α strains 13H10 (slice 1), 2B10 (slice 2), MUB3 (slice 3), MUC6 (slice 4), GST-QsbA (slice 5) and GST (slice 6), which contain plasmid clones or constructs p13H10, p2B10, pMUB3, pMUC6, pGST-QsbA, and pGST, respectively. Figure 1B shows results for bioassay of the indicated bacterial proteins GST-QsbA and GST.

[00020] Figure 2 is a graph showing the temperature and pH optimum profiles of AHL acylase.

[00021] Figure 3 is a graph showing the time course of OOHL inactivation by the purified AHL-acylase.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

[00022] A bacterial isolate of *Ralstonia sp.* XJ12B from a biofilm sample in a water treatment system was found to enzymatically inactivate AHLs, bacterial quorum-sensing molecules, in a bioassay using *Agrobacterium tumefaciens* strain Nt1 (*traR; tra::lacZ749*) as an indicator for AHL activity. The gene encoding the protein exhibiting this enzyme activity for AHL inactivation (*qsbA*) was cloned from a bacterial strain isolated from the biofilm sample and found to encode a peptide of 794 amino acids.

[00023] Bacterial cultures and bacterial proteins were assayed for the ability to inactivate AHL using *Agrobacterium tumefaciens* indicator cells. *A tumefaciens* was cultured at 28°C in MM medium as described in Zhang et al. (31). The bacteria or protein to be assayed is first mixed with an AHL substrate, for example N-β-oxooctanoyl-L-homoserine lactone (OOHL), and the reaction (inactivation of the AHL) is allowed to proceed. If AHL inactivation activity is present in the sample (*i.e.* the AHL has been cleaved and inactivated), then the inactivated AHL products fail to trigger the expression of *lacZ* reporter gene which is under the control of a TraR-dependent promoter. The strain *A. Tumefaciens* NT1 hosting the *lacZ* reporter system therefore does not turn blue in the presence of substrate 5-bromo-4-chloro-3-indolyl β-D-galactopyranoside (X-gal). See Example 2 for details of the bioassay. Any AHL may be used in the assay, as desired. Of course, any suitable assay for cleavage of AHL, including traditional *in vitro* enzyme assays may be used to detect the AHL inactivation activity. Those of skill in the art are able to modify or devise assays to detect and/or quantitate AHL inactivation.

[00024] *Escherichia coli* strain DH5a was used as a host for DNA manipulation. Both *Ralstonia sp.* and *E. coli* were cultured in LB medium (tryptone, 10 g/L, yeast extract, 5 g/L, and NaCl, 10 g/L, pH 7.0) at 37°C. Appropriate antibiotics were added when necessary at the following concentrations: ampicillin, 100 µg/ml; tetracycline, 10 µg/ml; and kanamycin, 20 µg/ml.

[00025] The gene encoding the protein responsible for the detected AHL inactivation was isolated using a cosmid library of 1600 clones with the genomic DNA of *Ralstonia sp.* strain XJ12B, constructed in *E. coli*. *E. coli* transfectants were screened for the ability to inactivate AHL. One clone, pl3H10, was found to inactivate AHL. Cosmid DNA from pl3H10 was digested, fused into a cloning vector, ligated and transformed into *E. coli*. The *E. coli* clones again were assayed for AHL inactivating activity. One clone, containing a 4 kb insert, had AHL inactivation activity.

[00026] Plasmids were subsequently purified for sequencing. The 4 kb fragment from clone p2B10 was completely sequenced according to known methods using ABI Prism dRhodamine Terminator Cycle Sequencing Ready Reaction Kit (Perkin-Elmer Applied Biosystems). See Table I, below. The sequence contained an open reading frame of 2385 nucleotides which was the AHL inactivation gene, *qsbA*, encoding a predicted polypeptide of 794 amino acids (85,373 Daltons).

Table I. *QsbA* Gene (*Ralstonia* sp.) Nucleotide Sequence (SEQ ID NO: 1).

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gtttgggaaaagtgggnagcgcgctgtgcag cgccccgcccctcagccgcgcagctcggcg cgcaccgaatgcgcgcgccggtgggcgccc 90
ggcggctggccggtgtggcgccggatcagg cgccggaaggcggacatgtcgtgataaccg cactgttcggcgattgccgtcaggctcagc 180
gtgctgacttccagcaggtggcaggcgcgc tccacgcgcagccggtgcagcaattgcagc ggcgaggtgcccagggtcttggtgaaatgc 270
cgcagcagcgtgcgctcgctggtcgaggcg gcggcggccagcttggccaggtcgaacggc tcgtgcaggtgctgctgcaggtagcgccgc 360
gcccgcagtaccacgctggtgcggatggcg ggcttgctgcgcagccagatggcggtggac tcaccgcgcgacgggtggtcgagcacggcc 450
tggccgagggtgcgtgccagccgggtgtcg gccaggcggccgaccaggcgctgcgtgagc gccacgccgtgctccatcgcgcgcgccgtc 540
agcacgttgccgctgctgacgatggcctgc tccgccaccaccttcagctgcgggtagttg ccgtgcagccagccggcgatcagccacgtc 630
accgtcaagcgccggccggcgggcagcgcg ccggccagcagcgccacgccggtgaaggac gaggccaccaagcctgccggcgtccaggta 720
gcgccggatggtggcgcgttcccactccag cagggccaggcgctgctccagcgtgctgat gtggtcgaaatgcaggggcgggacgaccag 810
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gcgcctgcgcgccgccgcaagactagagcg acacaagacaagaccgacaacaggagacaa cgcATGATGCAGGGATTCGCGCTGCGCGGC 1260
ACGCTCGCCATGGCCGCGCTCGCGGCGCTG GCCGGCTGCGCCAGTTCCACCGATGGCCGC TGGGGGTCGCTCAGCGACACCGGCCTGTCC 1350
GCCGAGATCCGCCCGCACCGGCTTCGGCATT CCGCACATCCGCGCCAACGACTACGCCAGC CTCGGCTATGGCATGGCCTATGCCTACGCG 1440
CAGGACAACCTGTGCCTGCTGGCCGACCAG GTGGTCACCGTCAACGGCGAGCGCTCGAAG ACCTTCGGGCCCGAGGGCACCGTGACGGTC 1530
TCGTTCAAGCCGATCCCCAACCTGCAGTCG GACGCCTTCTTCAAGGGCATCTTCGACGAG GACGGCCTGCGCGCCGGTTATGCGCAGATG 1620
TCGCCCGAGGCGCGCGAGCTGCTGCGCGGC TACATCGCCGGCTTCAACCGCTATCTCAAG GACACGCCGCCCGCCAACTTCCCGGCCGCC 1710
TGCCGCAATGCCGCCTGGGTGCGTCCGCTC ACGCTGGGCGACATGATGCGCATGGGCGAA GAGAAGGCGATCCAGGCCAGCGCCGGCGCC 1800
ATGCTGGCGGGCATCGTCGCCGCGCAGCCG CCGGGCCGCACGCCGGTGGCCGAGCGCGAG ATTCCGCCGCAGGCCGTCGACACCGTGGCG 1890
CTGGACCGCGAACTGCAGCTGCGCGACATG CCGATCGGCTCCAACGGCTGGGCCTTCGGC GCTGACGCCACCGCCAACCGGCGCGGCGTG 1980
CTGCTCGGCAATCCGCACTTCCCGTGGACG ACCACCAACCGCTTCTACCAGGTCCACCTG ACGGTGCCCGGCAAGCTCGACGTGATGGGC 2070
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TTGTTCGAACTGAAGCTGGCCGAAGGCGAC CCGACCACCTACCTGGTCGACGGCACGCCG CACAAGATGACCACCCGCACGGTCGCCTTC 2250
GACGTGAAGCTGCCCGGACGGCCGCCTCGAG CGCCGCACGCACACCTTCTACGACACCATC TACGGCCCGGTGCTGTCGATGCCGAGCGGC 2340
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CAGGCCCGGGACGTGGCCGGCATCCGCCAG GCCATCGGCAACCTGGGCATTCCCTGGGTC AACACCATCGCCACCGACCGCAACGGCCGC 2520
GCGCTGTTCGCCGACGTGTCGACCACGCCG GACGTCCCGGCCGCGGAGCTCCAGCGCTGT GCCCCGTCGCCGCTGGCCGGCAAACTCTTC 2610
AAGGACGCGGGCCTGGTGCTGCTCGACGGC TCGCGCGGCACCTGCAACTGGCAGGTCGAT CCGGCTTCGCCGGTACCCGGGCTGGTGGCG 2700
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GGCGACCTGCTCGCGGCATGCAAGGCCACG CCGGCCCCGGATGCCGACGTGCGCGACGGC TGCGCCGCCCTCGGCCAGTGGAACCGCACC 3060
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GGCGGCGAGGAATACGAAGGCGTGCTCAAC AAGCTGCAAACCCTGCCGATCGGGCCGAAG GGGCTGCCGGTGTATTTCGGCACCAGCTAC 3420
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TTCGACCAGATGCGTGCGTACTCGGGCAAG CACTGGAACCGGCTGCCGTTCTCCGAAGCG GCCATCGCGGCCGATCCGGCGCTGAAGGTG 3600
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gttggaaaccgcatccggacatgactgtat tgtgactctgcctgtgtccgtgt 3743
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The predicted open reading frame of the *qsbA* gene is shown in upper case letters with the start codon and stop codon in bold. A putative ribosome binding site (AGGAGA) is underlined.

[00027] Sequence analysis of this peptide indicated that *QsbA* did not have any significant homology with the known AHL-lactonase quorum-sensing molecule inactivator encoded by the *aiiA* gene from *Bacillus sp.* 240B1, however the deduced peptide sequence was typical of the primary structure of aculeacin A acylases (AACs) and penicillin G acylases, with signal peptide-α subunit-spacer-β subunit organization (16, 30). The *Ralstonia* sequence shares substantial identity with AACs from *Deinococcus radiodurans* strain R1, *Actinoplanes utahensis* and a putative acylase from *Pseudomonas aeruginosa*, all of which catalyze deacylation of their substrates. These AAC genes are translated as single precursor polypeptide and then processed to the active

form, which has two subunits. Aculeacin A is an echinocandin-type antifungal antibiotic with a long fatty side chain. Aculeacin A acylases purified from *A. utahensis* catalyze the hydrolysis of the amide bond on the palmitoyl side chain of aculeacin A (29). The primary structure of the protein, as well as enzyme activity analysis with different substrates, discussed below, therefore indicates that *qsbA* encodes an AHL-acylase which cleaves the amide linkage between the acyl side chain and the homoserine lactone moiety of AHLs.

[00028] The presumed α and β subunits of QsbA are located at amino acid positions 36-217 and 233-794, respectively, of SEQ ID NO: 2, with a 15 amino acid spacer between them, as determined by alignment with the peptide sequences from *D. radiodurans* strain R1, *A. utahensis* and *P. Aeruginosa*. See Table II.

Table II. Aligned Amino Acid Sequences of *QsbA* from *Ralstonia sp.* XJ12B (SEQ ID NO: 2), *D. radiodurans* strain R1 acylase (SEQ ID NO: 3), A. *utahensis* acylase (SEQ ID NO: 4) and *P. aeruginosa* acylase (SEQ ID NO: 5).

```
R.sp      MMQGF---ALRGTLAMAALAALAGCA-----SSTDGRWGSLSDTGLSAEIRRTGFGIPHIRANDYASLGYGMAYAYAQDN 72
D.rad     MSR-----SPFSSVSLPARLLLGSLL-----LGPLMLGGAASAQTYQVQIQRTAHGIPHIQASDLGGIGYGVGYSYAQDN 70
A.uta     MTSSY---MRLKAAAIAFGVIVATAA-----VPSPAS-GREHDGGYAALIRRASYGVPHITADDFGSLGFGVGYVQAEDN 71
P.aer     MSRPFRPPLCRETTSMGMRTVLTGLAGMLLGSMMPVQADMPRPTGLAADIRWTAYGVPHIRAKDERGLGYGIGYAYARDN 80
          *          : ::    :              .       . *: :..*:*** *.*  .:*:*:.*  *.**
                                          ↑
R.sp      LCLLADQVVTVNGERSKTFGPEGTVTVSFKPIPNLQSDAFFKGIFDEDGLRAGYAQMSPEARELLRGYIAGFNRYLKDTP 152
D.rad     LCLLADQVMTVRGERSKFLGAEGKTVVGFQPVNNLDSDVFFKTVIEPGRLQAGYRDQ-PQILALMRGYVAGVNRYLRDTP 149
A.uta     ICVIAESVVTANGERSRWFGATGPDDADVRTTSSTQAIDDRVAERLLEGPRDGVRAPCDDVRDQMRGFVAGYNHFLRRTG 151
P.aer     ACLLAEEIVTARGERARYFGSEGKSSAELD---NLPSDIFYAWLNQPEALQAFWQAQTPAVRQLLEGYAAGFNRFLREAD 157
           *::*:.::*..***:: :*. *   . .    .  :

R.sp      PANFP-AACRNAAWVRPLTLGDMMRMGEEKAIQASAGAMLAGIVAAQPPGRTPVAEREIPPQAVDTVALDRELQLRDMPI 231
D.rad     PEQWP-SACRNADWVRPLTELDVMRLGEEKAIQASAGAMVSAITSARPPQ----AGASTAAPRPDLAAFNRQYRFNDLPI 224
A.uta     VHRLTDPACRGKAWVRPLSEIDLWRTSWDSMVRAGSGALLDGIVAATPPT---AAGPASAPEAPDAAAIAAALDGTSAGI 228
P.aer     GKTTS---CLGQPWLRAIATDDLLRLTRRLLVEGGVGQFADALVAAAPPG----AEKVALSGEQAFQVAEQRRQRFRLER 230
              .   * .  *:*.::  *: *      :... * :  .:.:* **     *     .      .
                                                                  ↑———

R.sp      GSNGWAFGADATANRRGVLLGNPHFPWTTTNRFYQVHLTVPGKLDVMGASIAAFPVVSIGFNKDVAWTHTVSTGRRFTLF 311
D.rad     GSNGWAFGSEATTNGRGLLLGNPHFPWETSNRFYQLHLTLPGQFDVMGASLGGMPVVNIGFNQDVAWTHTVSTDKRFTLA 284
A.uta     GSNAYGLGAQATVNGSGMVLANPHFPWQGAERFYRMHLKVPGRYDVEGAALIGDPIIEIGHNRTVAWSHTVSTARRFVWH 288
P.aer     GSNAIAVGSERSADGKGMLLANPHFPWNGAMRFYQMHLTIPGRLDVMGASLPGLPVVNIGFSRHLAWTHTVDTSSHFTLY 290
          ***. ..*:: :.:  *::*.******  : ***::**.:**: ** **:: . *::.**..: :**:***.*  :*.
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R.sp      ELKLAEGDPTTYLVDGTPHKMTTRTVAFDVKLPDGRLERRTHTFYDTIYGPVLSMPSGGMPWTTQKAYALRDANRNNTRS 391
D.rad     ALTLVPGDPLSYVKDGQQRRLQRRTAVIEVKTANG-PRLHTRTVYFTPEGPLVNLPAAGLTWTPQYAFALRDANRNNTRM 383
A.uta     RLSLVPGDPTSYYVDGRPERMRARTVTVQTGSG-----PVSRTFHDTRYGPVAVVP-GTFDWTPATAYAITDVNAGNNRA 383
P.aer     RLALDPKDPRRYLVDGRSLPLEEKSVAIEVRGADGKLSRVEHKVYQSIYGPLVVWP-GKLDWNRSEAYALRDANLENTRV 390
           * *   **  *  **    :  ::...:.          :..: :  **:   * . : *.   *:*: *.*  *.*

R.sp      VDSWLHIGQARDVAGIRQAIG-NLGIPWVNTIATDRNGRALFADVSTTPDVPAAELQRCAPSPLAGKLFKDAGLVLLDGS 470
D.rad     LATWLGFAGAKSVRDIRASLN-VQGIPWVNTIAADRAGSALYADISSSPNVSAAQQQACTPPPLA-PLFPAAGLAVLDGS 461
A.uta     FDGWLRMGQAKDVRALKAVLDRHQFLPWVNVIAADARGEALYGDHSVVPRVTGALAAACIPAPFQ-PLYASSGQAVLDGS 461
P.aer     LQQWYSINQASDVADLRRRVEALQGIPWVNTLAADEQGNALYMNQSVVPYLKPELIPACAIPQLV-----AEGLPALQGQ 464
          .  *  :  * .*  ::  :     :****.:*:*  * **: : *  * :       *  . :        *   *:*.

R.sp      RGTCNWQVDPASPVPGLVAPARMPVLERDDYVANSNDSSWLTNPAQKLTGFSPVMGSVDVPQRLRTRIGLIEIGRRLAGT 550
D.rad     HSACDWKTDPASRVPGLRAPDKMPVLIRQDFVANSNNSAWLANPAAPQTGLDPLVGEVNAPQSPRTRMGLLEIGRRLSGT 541
A.uta     RSDCALGADPDAAVPGILGPASLPVRFRDDYVTNSNDSHWLASPAAPLEGFPRILGNERTPRSLRTRLGLDQIQQRLAGT 541
P.aer     DSRCAWSRDPAAAQAGITPAAQLPVLLRRDFVQNSNDSAWLTNPASPLQGFSPLVSQE-KPIGPRARYALSRLQGKQP-- 543
           . *    ** :  .*:  .  :**  * *:* ***:* **:.**    *:  ::..

R.sp      DGLPGNRIDLPNLQAMIFSNANLAGQLVLGDLLAACKATPAPDAD------VRDGCAALGQWNRTSNADA-RAAHLFREF 630
D.rad     DGLPGRTFDIPTLQATLLRESNLTGEMYAADAAKLCQS--AGGAE------LQPACNALAAWDRRSSQES-RGAALWREF 619
A.uta     DGLPGKGFTTARLWQVMFGNRMHGAELVRDDLVALCRRQPTATASNGAIVDLTAACTALSRFDERADLDS-RGAHLFTEF 621
P.aer     -------LEAKTLEEMVTANHVFSADQVLPDLLRLCRDN-QGEKS------LARACAALAQWDRGANLDSGSGFVYFQRF 613
                :   *   :  :   .:   *   *:       .     :  .* **. ::. :. ::  .   : .*

R.sp      WMRAKDIAQVHAVEFDPADPVHTPRGLR-MNDATVRTAVFKALKEAVGAVRKAGFALDAPLGTVQAAHAPDGSIALHGGE 702
D.rad     WRRARAIPNVYAVPFDPADPVNTPRGLN-TADPAAQTALLGALREAAAALTAAGIPFDAPLGEVQGVVRGGDFISLPGGA 691
A.uta     LAGG----IRFADTFEVTDPVRTPAPFWNTTDPRVRTALADACNGSPASPSTR------SVGDIHTDSRGERRIPIHGGR 690
P.aer     MQRFAELDGAWKEPFDAQRPLDTPQGIA-LDRPQVATQVRQALADAAAEVEKSGIPDGARWGDLQVSTRGQERIAIPGGD 686
                     *:   *: **  :     . . * :  *   : .            * ::        *.: **

R.sp      EYEGVLNKLQTLPIGPKGLPVYFG--TSYIQTVTFDDQGPVADAILTYGESTDHASPHAFDQMRAYSGKHWNRLPFSEAA 780
D.rad     EFEGVLDKIDFNPLAPGGYRGVVGNASSYIQTVGFTDSGVQAEAVLTYSQSSNPESPYFSDQTRLFSRSEWVKLPFTQPE 771
A.uta     GEAGTFNVITNPLVPGVGYPQVVHG-TSFVMAVELGPHGPSGRQILTYAQSTNPNSPWYADQTVLYSRKGWDTIKYTEAQ 769
P.aer     GHFGVYNAIQS--VRKGDHLEVVGG-TSYIQLVTFPEEGPKARGLLAFSQSSDPRSPHYRDQTELFSRQQWQTLPFSDRQ 763
             *. : :    :   .    .   :*:: * :   *  .  :*::.:*::  **   **   :* . *  : :::

R.sp      IAADPALKVMRLSQ--- 794
D.rad     IEADPTRTVVQLSE--- 785
A.uta     IAADPNLRVYRVAQRGR 786
P.aer     IDADPQLQRLSIRE--- 777
          * ***      : :
```

* = identical residues, : = conserved substitutions; . = semi-conserved substitutions; ↑ = post-translational processing sites for signal peptide and subunits; - = spacers.

[00029] The coding region of the *qsbA* gene was amplified by PCR. The amplified PCR products were digested, fused in-frame to the coding sequence of the glutathione S-transferase (GST) gene and expressed in *E. coli*. Protein extracts from the recombinant *E. coli* cells were assayed for the ability to inactivate AHL. Protein from *E. coli* expressing GST alone

served as a control. The results demonstrated that GST-QsbA fusion protein effectively eliminated AHL activity. See Figure 1B.

[00030] The substrate specificity of QsbA was determined by assaying total soluble protein extracted from the recombinant *E. coli* (pGST-QsbA) for inactivation of AHLs using substrates with acyl chains of differing lengths. QsbA was able to completely inactivate 3-oxo group acyl-HSLs having acyl chains of 8, 10 and 12 carbons. QsbA also strongly inactivated methylene group acyl-HSLs having acyl chains of 8 and 10 carbons. QsbA also inactivated the butyl and hexyl esters of N-β-octanoyl-L-homoserine, whereas the AHL-lactonase encoded by *aiiA* was unable to inactivate them. The substrate specificity data indicate that QsbA is an AHL-acylase.

[00031] QsbA and *qsbA* provide new tools for down regulation of AHL-mediated biological activities, such as the expression of virulence genes and biofilm differentiation in pathogenic bacteria, both *in vitro* and *in vivo*. The *qsbA* gene, which encodes the AHL inactivation enzyme (QsbA), or a functional fragment, subunit or substantially homologous variant thereof, may be introduced into a plant genome to produce a genetically modified plant with the ability to quench pathogen quorum-sensing signaling. Transgenic plants expressing an enzyme that inactivates AHLs can exhibit a significantly enhanced resistance to infection by bacterial pathogens, even when bacteria are present in high concentrations.

[00032] Methods of genetic manipulation and transformation of plant cells are well known in the art, as are methods of regenerating fertile, viable transformed plants. In general, any method of cloning the coding region of *qsbA* or a functional fragment or substantially homologous variant thereof into a suitable expression vector may be used. It is convenient to ligate the *qsbA* coding region into a vector, followed by ligation into a plant transformation vector, however those of skill are well aware of alternative methods to achieve the same results. Any suitable plant transformation vector may be used.

The vector contains the *qsbA* gene, or a functional fragment, subunit or substantially homologous variant thereof, so long as expression of the gene results in a QsbA protein or functional fragment, subunit or variant thereof which inactivates AHL.

[00033] A functional promoter preferably controls expression. Many suitable promoters are known in the art, such that a convenient promoter may easily be selected by a skilled artisan depending on the expression system being used. Such selection of a suitable promoter to achieve the desired level of translational expression is considered routine in the art. For example, it is advantageous to optimize *qsbA* expression by modification of codon usage and coupling to a strong promoter such as the double 35S promoter.

[00034] A suitable marker gene, such as kanamycin resistance, green fluorescent protein or any other convenient marker is advantageously used. Variations of the commonly used and well known methods for transforming plants with a gene, are well within the skill of the ordinary artisan in genetic manipulation of plants. Expression constructs may contain a signal sequence to direct secretion of the expressed QsbA protein, or may lack such a sequence, as desired. The plant transformation vectors containing the *qsbA* gene and a marker gene may be used to transform plant cells using *Agrobacterium*-mediated transformation. *Agrobacterium*-mediated transformation is conveniently used to transform plants with the *qsbA* gene, however any suitable method known in the art may be used, depending on the plant being transformed. For example, certain monocotyledonous plants are more efficiently transformed using other methods such as microprojectile bombardment, vacuum filtration or any other method known in the art to introduce and integrate DNA plasmids or fragments into the plant genome. Those of skill in the art are familiar with means to transform gymnosperms, monocots and dicots. All of these methods known in the art are contemplated for use with this invention.

[00035] After selection for transformants carrying the *qsbA* gene, transgenic plants may be regenerated according to known

methods in the art. Plants selected for a marker gene, for example kanamycin resistance, may be assayed, for example by PCR and DNA gel blot to determine how many copies of the *qsbA* gene are present in the plant tissue. Any suitable method known in the art is contemplated for use with the gene of this invention. QsbA enzyme activity may be detected in transgenic plants transformed with the *qsbA* gene by the bioassay method described in Example 2 or by any convenient method.

[00036] By "functional fragment, subunit or substantially homologous variant thereof," when referring to a *qsbA* nucleotide sequence, it is meant any fragment, subunit, variant or homologous sequence of *qsbA* (nucleotides 1234-3618 of SEQ ID NO: 1) which encodes a protein or peptide sequence capable of inactivating N-acyl homoserine lactones. "Substantially homologous variants" of a nucleotide sequence generally are those the complement of which hybridizes with *qsbA* under stringent or highly stringent conditions, for example temperatures of about 30°C to about 50°C, for example 30°C, 35°C, 37°C, 40°C, 45°C or 50°C, and/or salt concentrations of about 200 mM to about 1000 mM NaCl or the equivalent ionic strength, for example 200 mM, 250 mM, 300 mM, 400 mM, 500 mM, 750 mM or 1000 mM. The stringency conditions are dependent on the length of the nucleic acid and the base composition of the nucleic acid and can be determined by techniques well known in the art. Those of skill in the art are familiar with these conditions and ranges which are useful. Generally, a substantially homologous nucleotide sequence is at least about 75% homologous to SEQ ID NO: 1 or a fragment or subunit thereof, preferably at least about 85% homologous, and most preferably 90%, 95% or 99% homologous or more.

[00037] Those of skill in the art are familiar with the degeneracy of the genetic code, and thus are aware that nucleic acid sequences may be less than 100% homologous and yet encode the same protein or peptide sequence. Such variation in any of the sequences, fragments, subunits or substantially homologous variants also are contemplated as part of this invention.

[00038] Peptide and protein sequences which are encompassed by this invention include any sequences encoded by the *qsbA* gene, or any fragment, subunit or substantially homologous variant thereof. Such sequences therefore include any functional protein or peptide which retains the ability to inactivate AHL, including protein and peptide fragments of the complete QsbA protein, such as, for example, the sequences of amino acids 36-217 and 233-794 encoding by SEQ ID NO: 1 and substantially homologous variants thereof. A substantially homologous variant of the QsbA protein includes sequences which are at least about 50% homologous, preferably at least about 60% homologous, and most preferably 70%, 80% or 90% homologous or more. Therefore, a protein which is a substantially homologous varient of QsbA is about 50% to about 99.9% homologous with QsbA. Both conservative and non-conservative amino acid substitutions are contemplated, as well as sequences containing non-traditional or modified amino acids such as those known in the art.

[00039] The term "fragment" is intended to indicate any portion of the nucleotide of SEQ ID NO: 1 or protein/peptide sequence of SEQ ID NO: 2 which is greater than about 300 nucleotide bases or about 100 amino acids, up to one nucleotide or amino acid less than the entire sequence. The term "subunit" is intended to encompass any functional unit of the QbsA protein, such as, for example, amino acids 36-217 or 233-794 of SEQ ID NO: 2.

[00040] A protein or peptide sequence which is considered to inactivate N-acyl homoserine lactones is one which is capable of inactivating at least 55 pmoles N-acyl homoserine lactone (OOHL) per µg protein per minute at 30°C.

[00041] It has been previously demonstrated that quenching bacterial quorum sensing by inactivation of N-acyl homoserine lactone with AHL-lactonase stops bacterial infection (9, 10). The gene and protein described here, which is likely an AHL-acylase, represent a new and effective tool for inactivation of AHL signals and thus control bacterial infection. Similarly,

the gene and protein described here targets AHL quorum-sensing signals that regulate expression of pathogenic genes of many bacterial pathogens at a threshold concentration. This tool is applicable to all plant, animal or human diseases where the expression of pathogenic genes of bacterial pathogens is activated by AHL signals, such as, for example, plant pathogens *Erw. carotovora, Erw. Chrysanthemi, Erw. Stewartii;* human pathogens *P. aeruginosa, B. cepacia;* and animal pathogens X. *nematophilus, P. fluorescens* (1, 3, 6, 12, 17, 19, 22, 23, 24, 26).

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[00042] The following examples are provided to illustrate the invention described herein and should not be construed to limit the appended claims.

EXAMPLES

Example 1. Bacterial Isolation.

[00043] A bacterial biofilm sample was collected from a water treatment system and screened to isolate AHL inactivation bacterial strains. The bacterial mixture was suspended in sterilized water with shaking for 1 hour before spreading onto YEB medium (yeast extract, 5 g/l; casein hydrolysate, 10 g/l; NaCl, 5 g/l; sucrose, 5 g/l; $MgSO_4 7H_2O$, 0.5 g/l and agar, 15 g/l) plates. Individual colonies were restreaked on new plates to ensure purity of the isolates. Bacterial isolates were cultured in LB medium (tryptone, 10 g/L; yeast extract, 5 g/L, and NaCl, 10 g/L; pH7.0) in 1.5-ml Eppendorf™ tubes or 96-well plates at 28°C, with shaking, overnight, and assayed for AHL inactivation activity.

Example 2. AHL Inactivation Bioassay.

[00044] The bacterial culture to be assayed was mixed with an equal volume of fresh medium containing 20 μM N-β-oxooctanoyl-L-homoserine lactone (OOHL), or another AHL, when specified, to form a reaction mixture. The reaction mixture was incubated at 28°C for 4 to 5 hours, followed by 30 minute sterilization under UV light. Plates containing 20 ml MM agar medium (K_2HPO_4, 10.5 g/L; KH_2PO_4, 4.5 g/L; $MgSO_4 \cdot 7H_2O$, 0.2 g/L; $FeSO_4$, 4.5 mg/L; $CaCl_2$, 10 mg/L; $MnCl_2$, 2 mg/L; $(NH_4)_2SO_4$, 2.0 g/L; mannitol, 2.0 g/L; pH 7.0) supplemented with 5-bromo-4-chloro-3-indolyl β-D-galactopyranoside (X-Gal, 40 μg/ml) were prepared. The solidified medium was cut, still inside the plate, into separated slices (approximately 1 cm in width). See Figure 1. Five microliters of sterilized reaction mixture was loaded at the top of an MM agar strip, and then AHL indicator cells (*Agrobacterium tumefaciens* strain NT1 (*traR; tra::lacZ749*) (25) 0.7 μl cell suspension with an optical density at 600 nm of 0.4) were spotted at progressively further distances from the loaded samples. Plates were incubated at 28°C for 24 hours. A positive result for AHL inactivation is shown by the absence of blue colonies on the slice. A negative result for AHL inactivation is shown by the presence of blue colonies on the slice. For assay of protein for enzyme activity, total soluble bacterial protein was incubated with 20 μM of OOHL (or other AHL) at 37°C for 1 hour as the reaction mixture.

Example 3. Identification and Cloning the *qsbA* Gene.

[00045] Two bacterial isolates from the biofilm sample with distinct phenotypes, XJ12B and XJ12A, were found to possess the ability to inactivate AHL, with XJ12B showing stronger enzyme activity. The XJ12B isolate was cultured, centrifuged and sonicated. The strongest enzymatic activity was associated with the cell debris fraction rather than the soluble protein and supernatant fractions. These results indicated that the AHL inactivation activity is membrane associated. Sequencing of 16S rRNA was performed to identify the XJ12A and XJ12B isolates.

The 16S rRNA sequences of these isolates showed 97% and 96% identity, respectively, with that of *Ralstonia eutropha*.

[00046] To identify the gene encoding for AHL inactivation, a cosmid library of 1600 clones was constructed in *E. coli* with the genomic DNA of *Ralstonia sp.* strain XJ12B. Genomic DNA from the isolated *Ralstonia sp.* strain XJ12B was partially digested with Sau3A. The resulting DNA fragments were ligated to the dephosphorylated BamH1 site of cosmid vector pLAFR3 (28). The ligated DNA was packed with Gigapack IIIXL Packaging Extract (Stratagene) and transfected into *E. coli* DH5alpha. These *E. coli* transfectants were screened for AHL inactivation activity according to the methods described in Example 2 using OOHL as the substrate. Only a single clone (pl3H10) was identified as showing AHL inactivation activity (see Figure 1A, slice 1). To subclone the gene encoding the detected activity, cosmid DNA from the positive clone pl3H10 was partially digested with Sau3A and fused into BamH1 digested cloning vector pGEM-3Zf (+). The plasmids were ligated and transformed into *E.coli*, and the *E. coli* were assayed for the ability to inactivate AHL as described in Example 2. Clone p2B10, which contains a 4 kb insert, had AHL inactivation activity (see Figure 1A, slice 2). The TGS™ Template Generation System F-700 (Finnzymes OY) was used to mutate p2B10 plasmid DNA by randomly inserting the artificial Mu transposon, following the manufacturer's instructions. Plasmid clone p2B10, which contains the 4 kb insert containing the *qsbA* gene, was used as a template. Fifteen mutant clones were produced, and none was able to inactive AHL. Bacterial cultures of *E. coli* DH5α containing pMUG3 and pMUC6 are shown as examples in Figure 1A, slices 3 and 4, respectively. Plasmids were subsequently purified for sequencing using primers supplied in the kit.

Example 4. Sequencing and Sequence analysis of the *qsbA* Gene.

[00047] Sequencing was performed according to known methods using ABI Prism dRhodamine Terminator Cycle Sequencing Ready Reaction Kit (Perkin-Elmer Applied Biosystems). The 4 kb

fragment from clone p2B10 was completely sequenced and is shown in Table I. The sequence contains an open reading frame of 2385 nucleotides with an ATG start codon and a TGA stop codon (SEQ ID NO: 1, nucleotides 1259-3643). Based on the MU transposon mutagenesis data described in Example 3, this open reading frame is the coding region of the AHL inactivation gene, designated as *qsbA*. A putative ribosome binding site (AGGAGA) is located 6 base pairs upstream of the first ATG start codon (underlined in Table I).

[00048] The deduced peptide sequence shows the typical polypeptide primary structure of aculeacin A acylases (AACs) and penicillin G acylases, with signal peptide-α subunit-spacer-β subunit organization (16, 30). There are four additional potential start codons located 3, 36, 189 and 384 downstream from the first ATG. The longest open reading frame encodes 794 amino acids, with a predicted molecular weight of 85373 Daltons. The deduced peptide has 78 strongly basic and strong acid amino acid residues and a predicted isoelectric point of 7.48. The first 20 amino acid residues of the assumed open reading frame appear to be a signal peptide.

[00049] The peptide sequence of *qsbA* deduced from the open reading frame shares 40-52% identity with AACs from *Deinococcus radiodurans* strain R1, *Actinoplanes utahensis* and a putative acylase from *Pseudomonas aeruginosa*, The AACs' catalyze deacylation of their substrates. These AAC genes are translated as single precursor polypeptide and then processed to the active form of two subunits. By alignment with the peptide sequences from *D. radiodurans* strain R1, *A. utahensis* and *P. aeruginosa*, Table II, the presumed α and β subunits are located at amino acid positions 36-217 and 233-794, respectively, with a 15 amino acid spacer between them. *QsbA* shares less than 28% homology with penicillin G acylase (20) and cephalosporin acylase (21). See Table II. The amino acid sequence alignment in Table II was analyzed by the Clustal W program available from the European Bioinformatics Institute website (http://www.ebi.ac.uk).

Example 5. Expression of the *QsbA* Gene.

[00050] The coding region of the *qsbA* gene was amplified by PCR using a forward primer 5'-CGTGGATCCATGATGCAGGATTCGCCGCTGCGC-3' (SEQ ID NO: 6) and a reverse primer 5'- CGCGAATTCACCGGCAGCCCTCATGCGACAAC-3' (SEQ ID NO: 7) containing BamH1 and EcoR1 restriction sites, respectively. The amplified PCR products were digested using the above restriction enzymes and fused in-frame to the coding sequence of the glutathione S-transferase (GST) gene under the control of the isopropyl β-D-thiogalactopyranoside (IPTG) inducible lac promotor in vector pGEX-2T (Amersham Pharmacia) to generate construct pGST-QsbA. pGST-QsbA was transformed into *E. coli* and expressed.

[00051] Total soluble protein was extracted from the recombinant *E. coli* cells harboring the GST-QsbA-encoding fusion construct according to methods known in the art, based on the methods described in Dong et al. (9), and assayed for AHL inactivation. The total soluble protein from *E. coli* containing GST vector only was used as a control. For the bioassay, 50 µl of the soluble protein preparation (20 µg/µl) was added to the same volume of 40 µM AHL, *e.g.*, OOHL. After a 1 hour incubation at 37°C, the reaction mixture was assayed as described in Example 2. Representative data, shown in Figure 1B, slice 1, indicate that the soluble GST-QsbA fusion protein effectively eliminated OOHL activity.

Example 6. Characterization of the Substrate Spectrum of GST-QsbA Fusion Protein Expressed in *E. coli*.

[00052] To determinate the substrate spectrum of QsbA, total soluble protein extracted from the recombinant *E. coli* (pGST-QsbA) was assayed for inactivation of AHLs with acyl chains of differing lengths according to the methods of Example 2. The following AHLs were synthesized according to known methods as described by Zhang et al. (31): (1) N-octanoyl-L-homoserine lactone (C8HSL, OOHL); (2) N-decanoyl-L-homoserine lactone (C1DHSL, DHL); (3) N-β-oxohexanoyl-L-homoserine lactone (3-oxo-

C6HSL, OHHL); (4) N-β-oxohexanoyl-L-homoserine lactone (3-oxo-C12HSL, OdDHL); (5) N-β-oxohexanoyl-L-homoserine lactone (3-oxo-C8HSL, OOHL). The butyl and hexyl esters of N-β-oxohexanoyl-L-homoserine were prepared by esterification of N-β-oxohexanoyl-L-homoserine lactone with 1-butanol and 1-hexanol respectively, in the presence of small amount of Dowex 50H+ resin (Aldrich). The reaction was conducted at 60°C for 2 hours and the products were purified by silica column chromatography.

[00053] QsbA completely inactivated OOHL, N-β-oxodecanoyl-L-homoserine (ODHL) and N-β-oxododecanoyl-L-homoserine (OdDHL), which have acyl chains of 8, 10 and 12 carbons, respectively, at the concentrations tested (data not shown). QsbA also strongly inactivated N-β-octanoyl-L-homoserine(OHL) and N-β-decanoyl-L-homoserine(DHL), which have acyl chains of 8 and 10 carbons, respectively (data not shown). However, under the same reaction conditions, QsbA had less inactivating activity for N-β-oxohexanoyl-L-homoserine (OHHL), which has an acyl chain of 6 carbons (data not shown). The total soluble protein extract from control *E.coli* (pGST) did not show any activity against AHLs (data not shown).

[00054] QsbA also completely inactivated the butyl and hexyl esters of N-β-octanoyl-L-homoserine (data not shown). These two esters of N-β-octanoyl-L-homoserine showed comparable induction activity with OOHL when assayed with the AHL reporter strain *A. tumefaciens* NT1 (*traR; tra::lacZ749*) (data not shown). AHL-lactonase (encoded by *aiiA*) did not inactivate these substrates. These substrate specificity data are consistent with identification of QsbA as an AHL-acylase.

Example 7. Purification of AHL-acylase encoded by the *qsbA* gene

[00055] The GST-[AHL-acylase] fusion protein was purified using a glutathione Sepharose 4B affinity column following the manufacturer's instructions (Pharmacia). AHL-acylase was cleaved by digestion with thrombin (Sigma). Protein concentration was determined by measuring OD_{280}.

[00056] The purified AHL-acylase was incubated with OOHL for 20 minutes and the relative enzyme activity was measured by determining the residual OOHL in the reaction mixture, which contained 8 µM OOHL and about 0.6 µg AHL-acylase in a total reaction volume of 50 ml 1x PBS buffer. The reactions were stopped by addition of 1% SDS before loading on the assay plate. Determination of the OOHL activity was carried out in quadruplicate. AHL-acylase degraded OOHL in a range of temperatures from 22-42°C at pH 7.0. See Figure 2. The optimal temperature for enzyme activity was found to be approximately 28°C. Reaction temperature higher than 42°C decreased enzyme activity sharply. The optimal pH for enzyme activity also was determined. The AHL-acylase has a relatively narrow optimal pH range from pH 6.5 to 7.5. See Figure 2. The time course of OOHL inactivation by the purified AHL-acylase was determined at 30°C. See Figure 3. After 10 minutes, more than 82% OOHL had been degraded; the reaction rate was estimated to be about 55 pmols per µg AHL-acylase per minute.

CLAIMS:

1. A composition of matter which comprises a nucleic acid according to SEQ ID NO: 1.

2. A composition of matter which comprises a nucleic acid selected from the group consisting of nucleotides 1234-3618 of SEQ ID NO: 1, a fragment thereof and a substantially homologous variant thereof.

3. A nucleic acid according to claim 2 which comprises nucleotides 1234-3618 of SEQ ID NO: 1.

4. A composition of matter which comprises a peptidic sequence selected from the group consisting of a peptidic sequence according to SEQ ID NO: 2, a fragment thereof and a substantially homologous variant thereof.

5. A composition of matter which comprises a peptidic sequence encoded by a nucleic acid selected from the group consisting of nucleotides 1234-3618 of SEQ ID NO: 1, a fragment thereof and a substantially homologous variant thereof.

6. A composition of matter which comprises a peptidic sequence selected from the group consisting of SEQ ID NO: 2, a fragment thereof, a subunit thereof and a substantially homologous variant thereof.

7. A composition of matter according to claim 6 which comprises a peptidic sequence according to SEQ ID NO: 2.

8. A composition of matter according to claim 6 which comprises a peptidic sequence comprising amino acids 36-217 of SEQ ID NO: 2.

9. A composition of matter according to claim 6 which comprises a peptidic sequence comprising amino acids 233-794 of SEQ ID NO: 2.

10. A composition of matter according to claim 6 which inactivates AHL.

11. A method of modulating AHL signaling activity which comprises contacting said AHL with a composition of matter according to any one of claims 5-10.

12. A transgenic plant harboring a nucleic acid selected from the group consisting of nucleotides 1234-3618 of SEQ ID NO: 1, a fragment thereof and a substantially homologous variant thereof.

13. A transgenic non-human animal harboring a nucleic acid selected from the group consisting of nucleotides 1234-3618 of SEQ ID NO: 1, a fragment thereof and a substantially homologous variant thereof.

14. A method of controlling a bacterial disease in a mammal in need thereof which comprises administering to said mammal a composition of matter according to any one of claims 5-10, wherein the expression of pathogenic genes of said bacteria are regulated by AHL signals.

15. A method of claim 14 wherein said mammal is a human.

16. A method of controlling a bacterial disease in a plant in need thereof which comprises administering to said plant a composition of matter according to any one of claims 5-10, wherein the expression of pathogenic genes of said bacteria are regulated by AHL signals.

17. A method of controlling a bacterial disease in a mammal in need thereof which comprises administering to said mammal a

composition of matter of claim 2 and its peptide product, wherein the expression of pathogenic genes of said bacteria are regulated by AHL signals.

18. A method of claim 17 wherein said mammal is a human.

19. A method of controlling a bacterial disease in a plant in need thereof which comprises administering to said plant a composition of matter of claim 2, wherein the expression of pathogenic genes of said bacteria are regulated by AHL signals.

20. A method of controlling a bacterial disease in a plant using any bacterial species containing the composition of matter of claim 2.

RALSTONIA AHL-ACYLASE GENE

ABSTRACT OF THE DISCLOSURE

[00058] This invention provides a gene, *qsbA*, which encodes a protein useful for inactivating certain bacterial quorum-sensing signal molecules (N-acyl homoserine lactones) which participate in bacterial virulence and biofilm differentiation pathways. This gene was isolated from *Ralstonia sp.*, strain XJ12B. The invention also provides the QsbA protein, which possesses N-acyl homoserine lactone inactivating activity.

2577-154.PCT

Fig. 1

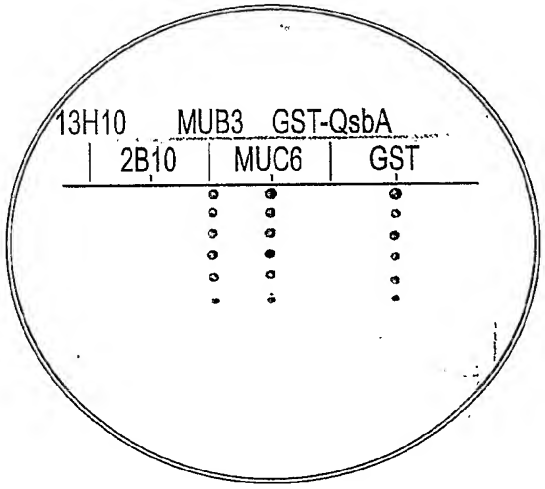

FIG. 1A

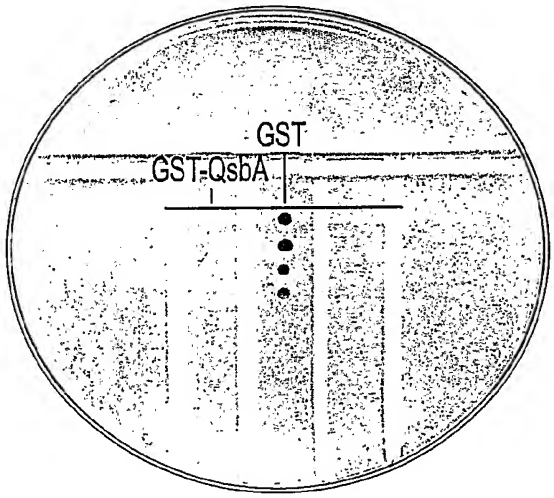

FIG. 1B

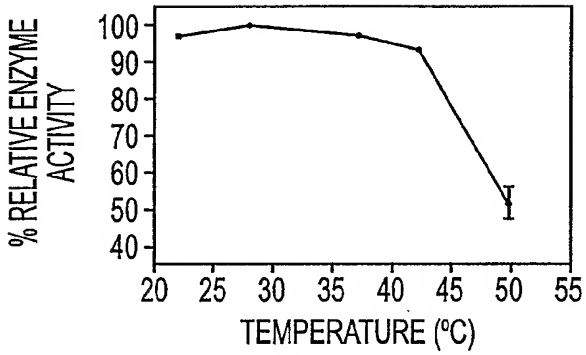

FIG. 2A

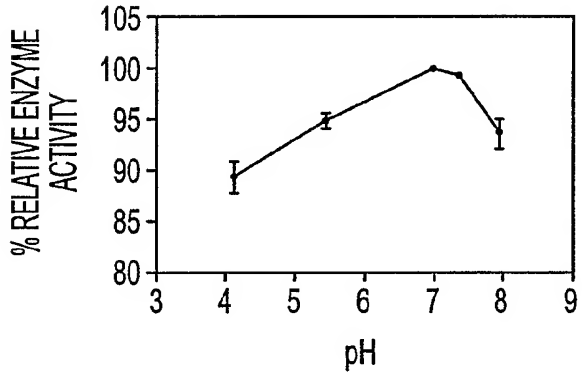

FIG. 2B

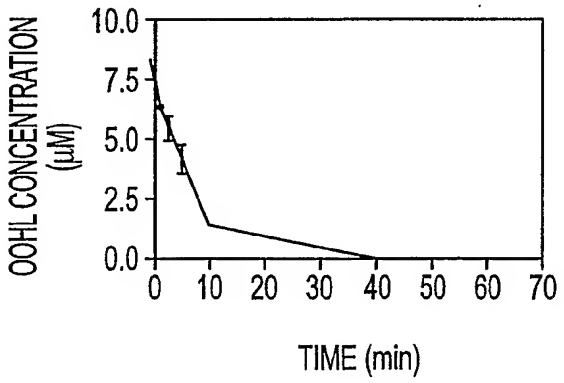

FIG. 3

SEQUENCE LISTING

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Xu, Jin Ling

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Thr Val Ile Glu Pro Gly Arg Leu Gln Ala Gly Tyr Arg Asp Gln Pro
        115                 120                 125

Gln Ile Leu Ala Leu Met Arg Gly Tyr Val Ala Gly Val Asn Arg Tyr
    130                 135                 140

Leu Arg Asp Thr Pro Pro Glu Gln Trp Pro Ser Ala Cys Arg Asn Ala
145                 150                 155                 160

Asp Trp Val Arg Pro Leu Thr Glu Leu Asp Val Met Arg Leu Gly Glu
                165                 170                 175

Glu Lys Ala Ile Gln Ala Ser Ala Gly Ala Met Val Ser Ala Ile Thr
            180                 185                 190

Ser Ala Arg Pro Pro Gln Ala Gly Ala Ser Thr Ala Ala Pro Arg Pro
        195                 200                 205

Asp Leu Ala Ala Phe Asn Arg Gln Tyr Arg Phe Asn Asp Leu Pro Ile
    210                 215                 220

Gly Ser Asn Gly Trp Ala Phe Gly Ser Glu Ala Thr Thr Asn Gly Arg
225                 230                 235                 240
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Gly Leu Leu Leu Gly Asn Pro His Phe Pro Trp Glu Thr Ser Asn Arg
                245                 250                 255

Phe Tyr Gln Leu His Leu Thr Leu Pro Gly Gln Phe Asp Val Met Gly
            260                 265                 270

Ala Ser Leu Gly Gly Met Pro Val Val Asn Ile Gly Phe Asn Gln Asp
        275                 280                 285

Val Ala Trp Thr His Thr Val Ser Thr Asp Lys Arg Phe Thr Leu Ala
    290                 295                 300

Ala Leu Thr Leu Val Pro Gly Asp Pro Leu Ser Tyr Val Lys Asp Gly
305                 310                 315                 320

Gln Gln Arg Arg Leu Gln Arg Arg Thr Ala Val Ile Glu Val Lys Thr
                325                 330                 335

Ala Asn Gly Pro Arg Leu His Thr Arg Thr Val Tyr Phe Thr Pro Glu
            340                 345                 350

Gly Pro Leu Val Asn Leu Pro Ala Ala Gly Leu Thr Trp Thr Pro Gln
        355                 360                 365

Tyr Ala Phe Ala Leu Arg Asp Ala Asn Arg Asn Asn Thr Arg Met Leu
    370                 375                 380

Ala Thr Trp Leu Gly Phe Ala Gly Ala Lys Ser Val Arg Asp Ile Arg
385                 390                 395                 400

Ala Ser Leu Asn Val Gln Gly Ile Pro Trp Val Asn Thr Ile Ala Ala
                405                 410                 415

Asp Arg Ala Gly Ser Ala Leu Tyr Ala Asp Ile Ser Ser Ser Pro Asn
            420                 425                 430

Val Ser Ala Ala Gln Gln Gln Ala Cys Thr Pro Pro Pro Leu Ala Pro
        435                 440                 445

Leu Phe Pro Ala Ala Gly Leu Ala Val Leu Asp Gly Ser His Ser Ala
    450                 455                 460

Cys Asp Trp Lys Thr Asp Pro Ala Ser Arg Val Pro Gly Leu Arg Ala
465                 470                 475                 480

Pro Asp Lys Met Pro Val Leu Ile Arg Gln Asp Phe Val Ala Asn Ser
                485                 490                 495

Asn Asn Ser Ala Trp Leu Ala Asn Pro Ala Ala Pro Gln Thr Gly Leu
            500                 505                 510

Asp Pro Leu Val Gly Glu Val Asn Ala Pro Gln Ser Pro Arg Thr Arg
        515                 520                 525

Met Gly Leu Leu Glu Ile Gly Arg Arg Leu Ser Gly Thr Asp Gly Leu
    530                 535                 540
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Pro Gly Arg Thr Phe Asp Ile Pro Thr Leu Gln Ala Thr Leu Leu Arg
545                 550                 555                 560

Glu Ser Asn Leu Thr Gly Glu Met Tyr Ala Ala Asp Ala Ala Lys Leu
                565                 570                 575

Cys Gln Ser Ala Gly Gly Ala Glu Leu Gln Pro Ala Cys Asn Ala Leu
            580                 585                 590

Ala Ala Trp Asp Arg Arg Ser Ser Gln Glu Ser Arg Gly Ala Ala Leu
        595                 600                 605

Trp Arg Glu Phe Trp Arg Arg Ala Arg Ala Ile Pro Asn Val Tyr Ala
    610                 615                 620

Val Pro Phe Asp Pro Ala Asp Pro Val Asn Thr Pro Arg Gly Leu Asn
625                 630                 635                 640

Thr Ala Asp Pro Ala Ala Gln Thr Ala Leu Leu Gly Ala Leu Arg Glu
                645                 650                 655

Ala Ala Ala Ala Leu Thr Ala Ala Gly Ile Pro Phe Asp Ala Pro Leu
            660                 665                 670

Gly Glu Val Gln Gly Val Val Arg Gly Gly Asp Phe Ile Ser Leu Pro
        675                 680                 685

Gly Gly Ala Glu Phe Glu Gly Val Leu Asp Lys Ile Asp Phe Asn Pro
    690                 695                 700

Leu Ala Pro Gly Gly Tyr Arg Gly Val Val Gly Asn Ala Ser Ser Tyr
705                 710                 715                 720

Ile Gln Thr Val Gly Phe Thr Asp Ser Gly Val Gln Ala Glu Ala Val
                725                 730                 735

Leu Thr Tyr Ser Gln Ser Ser Asn Pro Glu Ser Pro Tyr Phe Ser Asp
            740                 745                 750

Gln Thr Arg Leu Phe Ser Arg Ser Glu Trp Val Lys Leu Pro Phe Thr
        755                 760                 765

Gln Pro Glu Ile Glu Ala Asp Pro Thr Arg Thr Val Val Gln Leu Ser
    770                 775                 780

Glu
785
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<210> 4

<211> 786

<212> PRT

<213> A. utahensis

<400> 4

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            20                  25                  30

His Asp Gly Gly Tyr Ala Ala Leu Ile Arg Arg Ala Ser Tyr Gly Val
        35                  40                  45

Pro His Ile Thr Ala Asp Asp Phe Gly Ser Leu Gly Phe Gly Val Gly
    50                  55                  60

Tyr Val Gln Ala Glu Asp Asn Ile Cys Val Ile Ala Glu Ser Val Val
65                  70                  75                  80

Thr Ala Asn Gly Glu Arg Ser Arg Trp Phe Gly Ala Thr Gly Pro Asp
                85                  90                  95

Asp Ala Asp Val Arg Thr Thr Ser Ser Thr Gln Ala Ile Asp Asp Arg
            100                 105                 110

Val Ala Glu Arg Leu Leu Glu Gly Pro Arg Asp Gly Val Arg Ala Pro
        115                 120                 125

Cys Asp Asp Val Arg Asp Gln Met Arg Gly Phe Val Ala Gly Tyr Asn
    130                 135                 140

His Phe Leu Arg Arg Thr Gly Val His Arg Leu Thr Asp Pro Ala Cys
145                 150                 155                 160

Arg Gly Lys Ala Trp Val Arg Pro Leu Ser Glu Ile Asp Leu Trp Arg
                165                 170                 175

Thr Ser Trp Asp Ser Met Val Arg Ala Gly Ser Gly Ala Leu Leu Asp
            180                 185                 190

Gly Ile Val Ala Ala Thr Pro Pro Thr Ala Ala Gly Pro Ala Ser Ala
        195                 200                 205

Pro Glu Ala Pro Asp Ala Ala Ala Ile Ala Ala Ala Leu Asp Gly Thr
    210                 215                 220

Ser Ala Gly Ile Gly Ser Asn Ala Tyr Gly Leu Gly Ala Gln Ala Thr
225                 230                 235                 240
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Val Asn Gly Ser Gly Met Val Leu Ala Asn Pro His Phe Pro Trp Gln
245 250 255

Gly Ala Glu Arg Phe Tyr Arg Met His Leu Lys Val Pro Gly Arg Tyr
260 265 270

Asp Val Glu Gly Ala Ala Leu Ile Gly Asp Pro Ile Ile Glu Ile Gly
275 280 285

His Asn Arg Thr Val Ala Trp Ser His Thr Val Ser Thr Ala Arg Arg
290 295 300

Phe Val Trp His Arg Leu Ser Leu Val Pro Gly Asp Pro Thr Ser Tyr
305 310 315 320

Tyr Val Asp Gly Arg Pro Glu Arg Met Arg Ala Arg Thr Val Thr Val
325 330 335

Gln Thr Gly Ser Gly Pro Val Ser Arg Thr Phe His Asp Thr Arg Tyr
340 345 350

Gly Pro Val Ala Val Val Pro Gly Thr Phe Asp Trp Thr Pro Ala Thr
355 360 365

Ala Tyr Ala Ile Thr Asp Val Asn Ala Gly Asn Asn Arg Ala Phe Asp
370 375 380

Gly Trp Leu Arg Met Gly Gln Ala Lys Asp Val Arg Ala Leu Lys Ala
385 390 395 400

Val Leu Asp Arg His Gln Phe Leu Pro Trp Val Asn Val Ile Ala Ala
405 410 415

Asp Ala Arg Gly Glu Ala Leu Tyr Gly Asp His Ser Val Val Pro Arg
420 425 430

Val Thr Gly Ala Leu Ala Ala Ala Cys Ile Pro Ala Pro Phe Gln Pro
435 440 445

Leu Tyr Ala Ser Ser Gly Gln Ala Val Leu Asp Gly Ser Arg Ser Asp
450 455 460

Cys Ala Leu Gly Ala Asp Pro Asp Ala Ala Val Pro Gly Ile Leu Gly
465 470 475 480

Pro Ala Ser Leu Pro Val Arg Phe Arg Asp Asp Tyr Val Thr Asn Ser
485 490 495

Asn Asp Ser His Trp Leu Ala Ser Pro Ala Ala Pro Leu Glu Gly Phe
500 505 510

Pro Arg Ile Leu Gly Asn Glu Arg Thr Pro Arg Ser Leu Arg Thr Arg
515 520 525

Leu Gly Leu Asp Gln Ile Gln Gln Arg Leu Ala Gly Thr Asp Gly Leu
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Pro Gly Lys Gly Phe Thr Thr Ala Arg Leu Trp Gln Val Met Phe Gly
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Asn Arg Met His Gly Ala Glu Leu Val Arg Asp Asp Leu Val Ala Leu
                565                 570                 575

Cys Arg Arg Gln Pro Thr Ala Thr Ala Ser Asn Gly Ala Ile Val Asp
            580                 585                 590

Leu Thr Ala Ala Cys Thr Ala Leu Ser Arg Phe Asp Glu Arg Ala Asp
        595                 600                 605

Leu Asp Ser Arg Gly Ala His Leu Phe Thr Glu Phe Leu Ala Gly Gly
    610                 615                 620

Ile Arg Phe Ala Asp Thr Phe Glu Val Thr Asp Pro Val Arg Thr Pro
625                 630                 635                 640

Ala Pro Phe Trp Asn Thr Thr Asp Pro Arg Val Arg Thr Ala Leu Ala
                645                 650                 655

Asp Ala Cys Asn Gly Ser Pro Ala Ser Pro Ser Thr Arg Ser Val Gly
            660                 665                 670

Asp Ile His Thr Asp Ser Arg Gly Glu Arg Arg Ile Pro Ile His Gly
        675                 680                 685

Gly Arg Gly Glu Ala Gly Thr Phe Asn Val Ile Thr Asn Pro Leu Val
    690                 695                 700

Pro Gly Val Gly Tyr Pro Gln Val Val His Gly Thr Ser Phe Val Met
705                 710                 715                 720

Ala Val Glu Leu Gly Pro His Gly Pro Ser Gly Arg Gln Ile Leu Thr
                725                 730                 735

Tyr Ala Gln Ser Thr Asn Pro Asn Ser Pro Trp Tyr Ala Asp Gln Thr
            740                 745                 750

Val Leu Tyr Ser Arg Lys Gly Trp Asp Thr Ile Lys Tyr Thr Glu Ala
        755                 760                 765

Gln Ile Ala Ala Asp Pro Asn Leu Arg Val Tyr Arg Val Ala Gln Arg
    770                 775                 780

Gly Arg
785
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<211> 777

<212> PRT

<213> P. aeruginosa

<400> 5

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Met Met Pro Val Gln Ala Asp Met Pro Arg Pro Thr Gly Leu Ala Ala
35 40 45

Asp Ile Arg Trp Thr Ala Tyr Gly Val Pro His Ile Arg Ala Lys Asp
50 55 60

Glu Arg Gly Leu Gly Tyr Gly Ile Gly Tyr Ala Tyr Ala Arg Asp Asn
65 70 75 80

Ala Cys Leu Leu Ala Glu Glu Ile Val Thr Ala Arg Gly Glu Arg Ala
85 90 95

Arg Tyr Phe Gly Ser Glu Gly Lys Ser Ser Ala Glu Leu Asp Asn Leu
100 105 110

Pro Ser Asp Ile Phe Tyr Ala Trp Leu Asn Gln Pro Glu Ala Leu Gln
115 120 125

Ala Phe Trp Gln Ala Gln Thr Pro Ala Val Arg Gln Leu Leu Glu Gly
130 135 140

Tyr Ala Ala Gly Phe Asn Arg Phe Leu Arg Glu Ala Asp Gly Lys Thr
145 150 155 160

Thr Ser Cys Leu Gly Gln Pro Trp Leu Arg Ala Ile Ala Thr Asp Asp
165 170 175

Leu Leu Arg Leu Thr Arg Arg Leu Leu Val Glu Gly Gly Val Gly Gln
180 185 190

Phe Ala Asp Ala Leu Val Ala Ala Ala Pro Pro Gly Ala Glu Lys Val
195 200 205

Ala Leu Ser Gly Glu Gln Ala Phe Gln Val Ala Glu Gln Arg Arg Gln
210 215 220

Arg Phe Arg Leu Glu Arg Gly Ser Asn Ala Ile Ala Val Gly Ser Glu
225 230 235 240

Arg Ser Ala Asp Gly Lys Gly Met Leu Leu Ala Asn Pro His Phe Pro
245 250 255

Trp Asn Gly Ala Met Arg Phe Tyr Gln Met His Leu Thr Ile Pro Gly
260 265 270

Arg Leu Asp Val Met Gly Ala Ser Leu Pro Gly Leu Pro Val Val Asn
275 280 285

Ile Gly Phe Ser Arg His Leu Ala Trp Thr His Thr Val Asp Thr Ser
290 295 300

Ser His Phe Thr Leu Tyr Arg Leu Ala Leu Asp Pro Lys Asp Pro Arg
305 310 315 320

Arg Tyr Leu Val Asp Gly Arg Ser Leu Pro Leu Glu Glu Lys Ser Val
325 330 335

Ala Ile Glu Val Arg Gly Ala Asp Gly Lys Leu Ser Arg Val Glu His
340 345 350

Lys Val Tyr Gln Ser Ile Tyr Gly Pro Leu Val Val Trp Pro Gly Lys
355 360 365

Leu Asp Trp Asn Arg Ser Glu Ala Tyr Ala Leu Arg Asp Ala Asn Leu
370 375 380

Glu Asn Thr Arg Val Leu Gln Gln Trp Tyr Ser Ile Asn Gln Ala Ser
385 390 395 400

Asp Val Ala Asp Leu Arg Arg Arg Val Glu Ala Leu Gln Gly Ile Pro
405 410 415

Trp Val Asn Thr Leu Ala Ala Asp Glu Gln Gly Asn Ala Leu Tyr Met
420 425 430

Asn Gln Ser Val Val Pro Tyr Leu Lys Pro Glu Leu Ile Pro Ala Cys
435 440 445

Ala Ile Pro Gln Leu Val Ala Glu Gly Leu Pro Ala Leu Gln Gly Gln
450 455 460

Asp Ser Arg Cys Ala Trp Ser Arg Asp Pro Ala Ala Ala Gln Ala Gly
465 470 475 480

Ile Thr Pro Ala Ala Gln Leu Pro Val Leu Leu Arg Arg Asp Phe Val
485 490 495

Gln Asn Ser Asn Asp Ser Ala Trp Leu Thr Asn Pro Ala Ser Pro Leu
500 505 510

Gln Gly Phe Ser Pro Leu Val Ser Gln Glu Lys Pro Ile Gly Pro Arg
515 520 525

Ala Arg Tyr Ala Leu Ser Arg Leu Gln Gly Lys Gln Pro Leu Glu Ala
530 535 540

Lys Thr Leu Glu Glu Met Val Thr Ala Asn His Val Phe Ser Ala Asp
545 550 555 560

Gln Val Leu Pro Asp Leu Leu Arg Leu Cys Arg Asp Asn Gln Gly Glu
565 570 575

Lys Ser Leu Ala Arg Ala Cys Ala Ala Leu Ala Gln Trp Asp Arg Gly
580 585 590

Ala Asn Leu Asp Ser Gly Ser Gly Phe Val Tyr Phe Gln Arg Phe Met
595 600 605

Gln Arg Phe Ala Glu Leu Asp Gly Ala Trp Lys Glu Pro Phe Asp Ala
610 615 620

Gln Arg Pro Leu Asp Thr Pro Gln Gly Ile Ala Leu Asp Arg Pro Gln
625 630 635 640

Val Ala Thr Gln Val Arg Gln Ala Leu Ala Asp Ala Ala Ala Glu Val
645 650 655

Glu Lys Ser Gly Ile Pro Asp Gly Ala Arg Trp Gly Asp Leu Gln Val
660 665 670

Ser Thr Arg Gly Gln Glu Arg Ile Ala Ile Pro Gly Gly Asp Gly His
675 680 685

Phe Gly Val Tyr Asn Ala Ile Gln Ser Val Arg Lys Gly Asp His Leu
690 695 700

Glu Val Val Gly Gly Thr Ser Tyr Ile Gln Leu Val Thr Phe Pro Glu
705 710 715 720

Glu Gly Pro Lys Ala Arg Gly Leu Leu Ala Phe Ser Gln Ser Ser Asp
725 730 735

Pro Arg Ser Pro His Tyr Arg Asp Gln Thr Glu Leu Phe Ser Arg Gln
740 745 750

Gln Trp Gln Thr Leu Pro Phe Ser Asp Arg Gln Ile Asp Ala Asp Pro
755 760 765

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<212> DNA

<213> Artificial Sequence

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<223> Forward Primer for the QsbA gene

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<210> 7

<211> 32

<212> DNA

<213> Artificial Sequence

<220>

<223> Reverse Primer for the QsbA gene

<400> 7
cgcgaattca ccggcagccc tcatgcgaca ac 32

INTERNATIONAL SEARCH REPORT

International Application No
PCT/SG 02/00011

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N9/80 C12N15/57 A01K67/027 A61K38/50

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, BIOSIS, EMBL, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DATABASE SWISS-PROT 'Online! EBI-SBI; 1 May 2000 (2000-05-01) WHITE ET AL.: "Genome sequence of the radioresistant bacterium Deinococcus radiodurans R1; Science 286:1571-1577 (1999)" retrieved from SWISS-PROT Database accession no. Q9RYQ4 XP002208348 "Aculeacin A acylase from Deinococcus radiodurans R1; 53,316% identity with SEQ ID NO: 2 in 769 aa overlap". abstract --- -/--	4-6

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
14 August 2002	02/09/2002

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Ury, A

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	DATABASE EMBL 'Online! EBI; 23 November 1999 (1999-11-23) WHITE ET AL.: "Genome sequence of the radioresistant bacterium Deinococcus radiodurans R1; Science 286:1571-1577 (1999)" Database accession no. AE001836 XP002208349 "CDS complement (53991..56348): Encoding Aculeacin A acylase from Deinococcus radiodurans R1: 62,025% identity with SEQ ID NO: 1 in 1817 nt overlap". ---	2
X	WO 01 98214 A (NOVOZYMES BIOTECH INC) 27 December 2001 (2001-12-27) the whole document	2,4-6, 10-20
Y	same citations ---	2,4-6, 10-20
Y	LEADBETTER JARED R ET AL: "Metabolism of acyl-homoserine lactone quorum-sensing signals by Variovorax paradoxus." JOURNAL OF BACTERIOLOGY, vol. 182, no. 24, December 2000 (2000-12), pages 6921-6926, XP002208346 ISSN: 0021-9193 cited in the application abstract; figure 7 ---	2,4-6, 10,20
Y	LEADBETTER JARED R: "Quieting the raucous crowd" NATURE, vol. 411, 14 June 2001 (2001-06-14), pages 748-749, XP002208347 Figure 7 the whole document ---	2,4-6, 10-20
A	DONG YI-HU ET AL: "AiiA, an enzyme that inactivates the acylhomoserine lactone quorum-sensing signal and attenuates the virulence of Erwinia carotovora" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA, NATIONAL ACADEMY OF SCIENCE. WASHINGTON, US, vol. 97, no. 7, 28 March 2000 (2000-03-28), pages 3526-3531, XP002166712 ISSN: 0027-8424 cited in the application abstract --- -/--	1-20

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/SG 02/00011

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	DONG YI-HU ET AL.: "Quenching quorum-sensing-dependent bacterial infection by an N-acyl homoserine lactonase" NATURE, vol. 411, 14 June 2001 (2001-06-14), pages 813-817, XP001093866 cited in the application abstract	1-20

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/SG 02/00011

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 0198214 A	27-12-2001	AU 6993901 A	02-01-2002
		WO 0198214 A1	27-12-2001